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ANTIBACTERIAL ACTIVITY OF SEAWEEDS AGAINST SEAFOOD PATHOGENS

T. Vijayalekshmi[1] & F. Brisca Renuga[2]

[1]Research Scholar, Department of Zoology, Holy Cross College, Nagercoil, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

[2]Associate Professor, Department of Zoology, Holy Cross College, Nagercoil, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

ABSTRACT

Our study is to evaluate the potential bioactive compound of Seaweed and its antibacterial activity against Seafood pathogens.40 strains was active in antagonistic activity against various pathogens. Among the strains, antibacterial activity of seaweed species Gelidium spinosum Gracilariaedulis, Ulvalactuca, Ulvareticulata, Sargassummuticum, Gracilariagrevillea, Monostromalatismum, Kappaphycusalvarasi, Padinapavonica, Codiumfragile, Caulerpacorynephora, Leathesiamarina, Padinatetrastromatica, Laurenciakarachiana, Sargassumweightii, Porphyraumbilicalis, Colpomeisisinosa showed better activity against Escherichia coli. The inhibition zone of red algae and brown algae give maximum effect. Sargassum wightii seaweed Acetone extracts 3.5mm against Escherichia coli. Laurenciakarrachiana seaweed Acetone extract 3.0mm zone of inhibition against Escherichia coli.

KEYWORDS: *Marine Algae, Seafood Pathogens, Antibacterial Activity, Antagonistic Compound*

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INTRODUCTION

Marine macroalgae which are popularly termed as sea weeds belong to the primitive group of non- flowering plants known as Thallophyta.

They are autotrophic plants and grow in the intertidal and sub -tidal regions of the Sea. They grow abundantly where ever rocky or Coral substratum is available for their attachment with the help of Rhizoids or hold fast.

Selective utilization of marine algae as potential source of pharmaceutical agents has been increasing in recent years. Many of the seaweeds possess bioactive components which inhibit the growth of some of the Gram positive and Gram negative bacterial pathogens. The algal extracts were used as curative and preventive agents for various diseases such as Cancer, antibiotics, antihelmintics, Coughremedies, antihypertensive, antitumour and antidiarrhoea. Recently we have embarked on the chemical investigative of marine algae with a special accent on their bioactive properties.

Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromaics, nitrogen-heterocyclic, nitrosulphuric -hetrocyclic, sterols, bibutanoids, proteins, peptides and sulphated poly saccharides. The crude extract thus obtained is subjected to broad based biological screening for antifungal, antiviral, antibacterial,

antimalarial, antifilarial, hypoglycaemic and antifertility activity. On the other hand, the algae are also used as food stuff, animal fodder, fertilizer, industrial material such as agar and minor medicines. The antimicrobial activity of the selected species of marine algae was not uniform.

Seaweeds is a multi cellular species, commonly subdivided into red, green, blue green algae, and brown algae due to their pigmentation according to Phyla Rhodophyta, Heterocontophyta and Chlorophyta. The Seaweeds are widely utilized in food, Medicine, Fertilizer and Bioethanol production. The chemical composition of seaweeds is Carbohydrates, Proteins, Minerials, Lipids and so on.

Seaweeds contain 60 trace elements whose concentration is higher than terrestrial plants. Sea weeds the only source of Agar-Agar, Algin, and Carrageenan-phyto chemicals that have wide application in foods, confectionary, pharmaceuticals, dairy and paper industries as gelling, stabilizing and thickening agents. In India -Andoman and Nicobar islands and Lakshadweep have rich resources of Sea weeds.

The total resources of Seaweeds in India are about 70, 000 tonnes. Seaweeds are not plants and so lack true leaves, stems, and roots.

Types of Seaweeds (Salt Water)

- Phaeophyceae-Brown algae
- Chlorophyceae-Green algae
- Rhodophyceae-Red algae

Seaweeds have a challenging life on the seashore where it has to survive in salty water, CrashingWaves, Tides and exposure to the heat and Sun.

The complete body is known as the thallus whether it is a filament, a thin leafy sheet.

Sea weeds provides an important source of food and protection for a wide variety of marine animals, as well as generating life and providing oxygen in the water.

Seaweeds regulates and control moisture loss.

Seaweeds forming is one of the top priorities so for development in Malaysia due to the increasing world demand for processed Seaweed.

Seaweed farming has been identified as one of the high impact Aquaculture activities in Malaysia due to the increasing World demand for raw and processed Seaweed with reported global World demand in 2012 of about 3, 50, 000 to 4, 00, 000 metric tonnes.

Seaweeds from Marine resources often consists of maximum amount of Carbohydrates(poly sacchrarides), Alginate and Fucoiden from brown algae, Carrageenan from red algae, and Ulvan from green algae are gaining considerable attentions for individual application (drug delivery and tissue engineering).

In broiler production, feed cost constitutes the largest operation cost, with this, many researchers focused their attention on how to reduce feed cost to realize a larger profit. The use of locally available materials as feed added to commercial feeds broiler ration may be one solution to lower feed cost. Seaweeds had been used for many years directly

for human consumption and animal feed. It is also an ingredient for the global food and cosmetics industries and is used as fertilizer and as a animal feed supplements. Also, seaweeds are valuable sources of micro food nutrients and raw materials for the pharmaceuticals industry.

Seaweeds has plenty of essential nutrients especially trace elements and several other bio active substances. That explains why today seaweeds are considered as the food supplement for the 21st centuries as a source of proteins, lipids, polysaccharides, minerals, vitamins and enzyme. Sargassummuticum is a brown edible algae extract have various biological activities including antioxidant, antimicrobial, and anti inflammatory properties.

Seaweeds have been used in poultry to improve animal immune status to decrease microbial load in the digestive tract and for their beneficial effect on quality of poultry meat and eggs.

Marine algae are not only the primary and major producers of organic matter in the Sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. They contain compounds ranging from Sterols, Terpenoids, to brominated phenolic, which shows bioactive against microorganisms. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antiviral, antifungal, antineoplastic, antifouling, antiinflamatory, antitumoric, cytotoxic and antimitotic activities. Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideasfor the development of new drugs against Cancer, micro bialinflamations and it is an universally known fact that marine algae has got rejuvenating properties where they have been used as source of nutrients in many countries. Seaweeds are plant -like ocean organisms that are botanically classified as macrophytic marine algae. Edible Seaweeds are often called.”Seavegetables”. Seaweeds come in an amazing variety of beautiful shapes, colours and sizes, and are found in all of the world’s Oceans.

Seaweed has been a part of diets in China, Japan and Korea. New Zealand tradionally used a few species of red and green seaweed.

Seaweeds contains high levels of Iodine, Calcium, and Magnesium. Seaweed as possible source of biologically - active Vitamin B12.In Seaweeds one study 2014 noted that B12 was found in both raw and roasted Seaweed.

As a Nutraceutical product, some edible Seaweeds are associated with anti inflammatory, anti-allergic, antitumor, a extraction of major compounds from the antidiabetic, antioxidant, antihypertensive, and neuro protective properties. They have been screened extensively to isolate life saving drugs or biologically active substances allover the world. The revolutionized therapy of infectious diseases by the use of antibacterial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produced. These limitations demand for improved pharmacokinetic properties which necessitate the continued research for new antibacterial compounds for the development of drugs. The extraction of major compounds from the different species of Seaweeds was solvent dependent. There are a lot of reports from around the world related to that Seaweed species were extracted using organic solvents.

Seaweeds are of nutritional interest as they low calorie food, but rich in Vitamins, minerals, and dietary fibres. In addition to vitamin and minerals, seaweeds are also potentially good source s of proteins, polysaccharides, and fibres. The lipids which are present in very small amounts are unsaturated and afford protection against cardiovascular pathogens.

Seaweeds extracts can become an alternative eco-friendly approach for disease control in aquaculture (Romero etal 2012).

Commercial cultivation of the red alga Kappaphycusalvarezi, (Doty) has been satisfying the demand of the Carrageen an industry for more than 40 years. The present review focuses on Kappaphycus forming techniques through the application of biotechnological tools, ecological interactions with endemic ecosystems, future Kappaphycusalvarezii forming potentials in Asia, Africa and the Pacific and the challenges for prospective farmers. The introduction of Kappaphycus cultivation to tropical countries will continue due to the high production values realized, coastal villages searching for alternative livelihoods, and the increased global industrial demand for Carrageen an.

The study of seaweed is known as Phycology. They belong to three different groups, empirically distinguished since the mid -nineteenth century on the basis of thallus colour.

Seaweed is used in worldwide it contains vitamins, minerals and fiber. Seaweeds have curative powers for the treatment of Tuberculosis, Arthritis, Cold, and Influenza. Seaweeds broadly says algae as a cheap source in the preparation of new chemicals. Seaweeds played a milestone in pharmaceutical industries. The ancient Romans used them to treat wounds, burns and rashes.

Seaweeds are found attached to the solid bottom substrate of rocks, dead corals, pebbles, shells and other plant materials. The seaweeds are found in relatively shallow coastal waters, estuaries, intertidal and deep sea areas up to 180 meters depth. About 40, 000 tonnes of seaweeds are being exploited for the production of algin and agar every year. Review on the seaweed resources, cultivation and its utilization from different Indian coastal water has well documented by Subba Rao.

Seaweeds are also harvested or cultivated for the extraction of polysaccharides such as Alginate, Agar, and Carrgeenan, gelatinous substances collectively known as hydrocolloids or phycocolloids. Hydrocolloids have attained commercial significance, especially in food production as food additives. The food industry exploits the gelling, water -retention, emulsifying and other physical properties of these hydrocollods.

Most marine macroalgae are non-toxic in normal quantities, can have a laxative and electrolyte- balancing effect. It’s extremely versatile and can be used in many dishes, including Sushi, Rolls, Soups, and Stews, Salads. Seaweed is highly nutritious, Science -backed benefits of seaweed, contains Iodine and Throsine which support Thyroid function, your thyroid gland releases hormones to help control growth, energy production, reproduction and the repair of damaged cells in your body. Seaweed also contains an amino acid called Tyrosine, which is used alongside iodine to make two key hormones that help the thyroid gland do its job properly.

Seaweeds contains small amounts of vitamins A, C, E, and K along with folate, Zinc, Sodium, Calcium and Magnesium. Seaweed can also be a good source of Omega 3 fats and vitamin B12.

Antioxidants can make unstable substances in your body called free radicals less reactive. These have been shown to protect your body, s cells from free radical damage. Particular Carotenoid called Fucoxanthin, the main Carotenoid found in Brown algae, Fucoxanthin has been shown to protect cell membrances. Seaweed contains fiber and sugars, both of which can be used as food sources for the bacteria in your gut. The fiber can also increase the growth of good bacteria and nourish your gut.

Seaweed is also considered to have anti-obesity effects. Fucoxanthin may help reduce body fat. Seaweeds may help reduce your blood cholesterol levels lower total cholerol, lower LDL-cholesterol and triglyceride levels.

Heart disease can also be caused by excessive blood clotting. Seaweed contains carbohydrate s called fucans, which may help prevent blood from clotting. Fucans extracted from seaweed prevented blood clotting as effectively as a anti-clotting drug.

Diabetes is a major health problem. By the year 2040, 642 million people worldwide are expected to have type 1 or type 2 Diabetes. Brown seaweed contains fucoxanthin; alginate may reduce blood glucose level consequently reducing your risk of Diabetes.

Seaweeds, some potential dangers of consuming too much. Seaweeds can contain a very large and potentially dangerous amount of Iodine. Seaweed can also accumulate heavy metals, but this is not considered a health risk.

Seaweed is water -soluble, which means cooking and processing it can affect its iodine content.

Seaweeds are of nutritional interest as they contain low calorie food, but rich in vitamins, minerals and dietary fibres. In addition to vitamin and minerals, seaweeds are also potentially good sources of proteins, polysaccharides, andfibres. The lipids which are present in very small amounts are present in very small amounts are unsaturated and afford protection against Cardiovascular diseases.(LDL-Bad cholesterol).

Seaweed may also help protect you from certain types of infections. Plant compounds may have the ability to fight viruses such as Herpes and HIV by blocking their entry into cells. Seaweeds may have some beneficial effects on your immune system. Seaweeds also contain agars, carrageenans and fucoidans which are thought to act as prebiotics. Prebiotics are a type of non digestible fiber that feed the beneficial bacteria in your gut. The soluble fiber found in seaweed may also help protect against the development of colon cancer. Some compounds found in brown varieties may help prevent the spread of cancerous cells.

Skin Damage

Compounds in seaweed may help protect the skin from damage caused by UV rays from the Sun.They may also help prevent wrinkles, Sun spots and premature skin aging.

Bone and Inflammatory Diseases

Seaweeds antioxidant and anti inflammatory effects may help reduce the risk of Rheumatoid arthritis and Osteoporesis.

Cancer

Brown seaweed contains an element called Fucoidan, shown promise in eradicating or slowering the spread of colon rectal and breast cancers.

Oxidative damage can harm your DNA and cells Seaweeds can protect against oxidative damage.

One study examined 87 people from India with precancerous lesions called oral sub mucoid fibrous in the mouth, amoung those who took 1 gram of Spirulina per day for one year, 45 % saw the lesions disappear.

Allergic rhinitis is charactized by inflammation in your nasal passageways. It is triggered by environmental allergens, such as pollen, animal hair or even wheat dust., 2 grams per day dramatically reduced symptoms like nasal discharge, sneezing, nasal congestion and Itching.

Caulerphasps they have also been used to treat some diseases like Cancer, Arthritis etc.

Figure 1: Picture Shows Ocean Receding at Porbandar Beach.

Figure 2: Coastal Line Around Gujarat.

Porbandar is My Study Area for Seaweed Collection.

Figure 3: Herbarium Morphology of Laurencia Karachiana.

Figure 4: Ulva Reticulata Seaweed Morphology.

Seaweed Fermentation Using Lactic Acid Bacteria Were Selected Local Seaweeds

Sargassum Sps

Ulva Lactuca

As sole source in the growth media. Seaweeds fermentation according to their ability to grow and produce organic acids. The optimum seaweeds fermentation period was determined by monitoring the fermented samples at regular interval for a period of 5 weeks during which activated partial thromboplastin time (APTT). Prothromb in time(PT)as well as antioxidant provides information to pave a way towards the development of wide range seaweed functional foods.

Pathogenic bacteria in water bodies cause heavy mortality in wild as well as cultured aquatic animals. These problems are usually tackled by following preventive methods or by treating animals with drugs or chemicals. Due to increased number of diseases, the use of antimicrobial agents has increased significantly in aquaculture practices(Burridge et al., 2010).Due to indis criminate use of antibiotics to evade from diseases in aquaculture, the microorganisms have developed new drug-resistant bacterial strains(Perez et al., 2016).Hence developing cheaper and effective natural antimicrobial agents with better potential, less side effects than antibiotics, good bioavailability and minimal toxicity is necessary (Thanigaivel et al., 2015).Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas(Shelar et al., 2010), and are already in use as traditional medicine for along time (Taskin et al., 2007).Hence they have recently received significant attention for their capacity to provide a rich source of primary and secondary metabolites(Tuney et al., 2006).Which have been characterized as a broad spectrum of anti bacterial agents (Cox et al 2010);(Lavanya & Veerappan, 2011);antiviral (Gomez et al., 2010).anticancer compounds(Boopathy and Katheresan, 2010)antioxidant compounds (Vinayak et al., 2011)antifouling compounds(Manilal et al., 2011) and pharmaceutical preparations (Yuvaraj et al 2011).Seaweeds are also used in agricultural seed treatment for organic farming practice(Ambika and Sujatha., 2016).Seaweeds have been recoganized as potential sources of the antibiotic substances (Shimaa et al., 2016)Among them fattyacids, terpenes, carbonyls and bromo-phenol compounds in seaweeds are responsible for antibiotic activity (Aubert et al., 1979).

The ancient Romans used them to treat wound, burns, and rashes. Egyptians may have used them as a treatment for breast cancer. Seaweeds posses' powerful cancer -fighting agents that will eventually prove effective in the treatment of malignant tumors and leukemia in people. These versatile marine algae have also contributed to economic growth. Among their many uses in manufacturing, they are effective binding agents (emulsifiers) in such commercial goods as toothpaste and fruit jelly, and popular softeners (emollients)in organic cosmetics and skin-care productions.

Many natural products from these organisms act as chemical weapons and are highly potent inhibitors of physiological processes in prey, predator or competitor. Several show pharmacological activities and are effective against Cancer, AIDS, Arthritis. The marine flora-based anti cancer research in the present context of increasing cancer incidence, deprived of the cheaper, safer, and potent medicines to challenge the dreadful human disease. The antioxidants may be able to cause the regression of prematignant lesions and inhibit their development into cancer. The phyto chemicals possibly activate macrophages, induseapoptosis, and prevent oxidative damage of DNA, thereby controlling carcinogenesis. The marine floras are largely unexplored for anticancer lead compounds. Majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. The alkaloids present in marine algae may be divided into 3 groups. Phenylethylamine, alkaloids, in dole and halogenated in dole alkaloids. Grenha et al fabricated carrageen an one of the marine derived natural polymer. Laminaria seaweed thin sticks used as abortion medicine.

MATERIALS AND METHODS

Collection of the Seaweed

Five species of green algae, seven species of red algae, sixspecies of brown algae were collected in different seasons November 2019 to April 2021.All of the seaweeds were collected manually in the intertidal zone region of the Gujarat coast, and preserved on ice and voucher specimens were frozen at -20°c for identification and future reference. The seaweeds were washed thoroughly with sea water to remove epiphytic and extraneous materials and brought to the laboratory in plastic bags. Then samples were shade dried for one month, ground in an electric mixer or using morter and pestle and stored in refrigerator at 4°c.

Identification of the Seaweed

The study area, comprising of numerous sandy beaches and irregularly distributed rocky substratum interspersed with sandy intertidal pools inhabited with a wide variety of marine algae, Subash nager, Porbander, Gujarat, India. I have prepared variety of seaweed species in a form of Herbarium. The morphological and anatomical characteristics of all species were observed under a zoom stereo Microscope. The peculiar characteristics taken into account include lenth and diameter of the rhizome, its internal structure, distribution of the rhizome length, width of the assimilator, colour, diameter of the peltate head, length of the lateral branches, size and shape of primary, secondary, and tertiary leaves, size and shapes of air bladders, branching and lenth of receptacles, shape and size of holdfast, reproductive characters like, spermata, tetrasporangia and cystocarp.

Preparation of Extracts

The seaweeds were rinced with sterile sea water to remove any adherents, and necrotic parts, and then dried in the shade at room temperature.The dried seaweeds were then powdered in a electric grinder or using sterile morter and pestle.Each powdered sample of seaweeds(2g) each suspended in 3 different solvents(methanol, acetone and distilled water)in 50ml respectively. After 72 hrs the mixture was kept in shaker 25°c and 100 RPM for 9 hrs.The filtrate was filtered through whatman no 1 filter paper fitted with a buchner funnel using suction presser funnel or glass funnel, the process was repeated once more and the two filtrates were combined. The collected filtrates were stored in the refrigerator for future further activity studying the extract were tested for their antimicrobial activity on clinical isolates.

Antibacterial Activity Assay

Isolation and Identification of Pathogens

Samples were collected from the infected marine products using sterile swab. After the collection of pathogenic bacteria from sample they were lawn cultured on the Tergital- 7 agar, Thiosulphate citrate bile salt sucrose agar, Blood agar, Xylose lysine deoxycholate agar selective agar medium.

After incubation the morphology and biochemical activities of the colonies were observed and identified as Escherichia coli, Enterobacteraerogenes, Staphylococcusaureus, Vibriocholera, Vibrioparachemolyticus.

BIOCHEMICAL TEST

Gram, s Staining

Prepare a smear, put a drop of sterile saline, aseptically add a little of the colony for staining (18 -24 hours culture) mix well in the saline, air dry, fix by passing the slide 2-3 times through a Bunsen flame. Flood the smear with crystal violet for 1 minute. Wash with tape water. Flood the smear with crystal violet for 1 minute. Wash with tape water. Flood the smear with gram, s iodine for 1 minute. Wash with ethyl alcohol (75%).Wash with tape water.

Flood with Safranin for 30 seconds. Wash with tape water. Airdry. Observe under Microscope. Gram-positive organisms stain violet colour.

Gram-Negative organisms stain red colour.

Motility Test

Tube Method

Inoculate motility medium by stabbing into the top of a tube of the semi solid medium to a depth of about 5mm.Incubate at 35-37°c for 48 hrs and observe for spreading of the growth through the medium.

Slide Motility Method

Hanging Drop Method

Place vasaline in four corners of a cover slip. Using a sterile loop place a drop of an 18 hrs nutrient broth culture centre of the cover slip. Place a clean microscope slide (cavity slide) over the top of the cover slip. Invert the slide so that the drop is upside down. Observe under microscope using x 40 objectives. The bacterium can be said to be motile when it is seen to move from one side of the field of view to the other.

Indole Test

Inoculate tubes of Tryptone broth incubate tubes at 37°c for 24 hours. Add 0.2-0.3 ml of in dole (Kovac, s) reagent to the tube and shake. Allow 10 minutes and observe the results. A dark red colour in the amyl alcohol surface layer constitutes a positive test.

Methyl Test

Inoculate tubes of MRVP medium inoculate tubes at 37°c for 5 days. Add five drops of methyl red solution and shake. Record a distinct red as methyl red positive, a distinct yellow as methyl red negative.

Voges -Proskauer Test

Inoculate tubes of MRVP medium and incubate at 37°c 48 hrs. Pipette 1 ml of each culture to $_4$ a separate empty culture tube and add 0.6 ml of α-naphthol solution and 0.2ml of potassium hydroxide solution. Shake the tubes and let them stand 2-4 hours.Record the development of pink colour in the mixture as a positive test.

Simmon's Citrate Agar

Inoculate in Simmon's Citrate agar slant and slab the butt and incubate for 96 hrs at 37°c.Usually accompanied by colour change from green to blue.

Catalase Test

Place 30 % Hydrogen Peroxide (H_2 O_2) in a clean slide. Add an 18 hrs young culture use platinum loop and mix well, Bubbles formation in slide means Positive reaction. If bubbles are absent Negative reaction.

Oxidase Test

Place a piece of filter paper into an empty petridish and add 3 drops of tetramethyl paraphenylene diamine dihydrochloride solution to its centre with a sterile glass rod smear cells thoroughly into the reagent. The oxidase test is positive if transferred cells turn dark purple in5-10 seconds.

Urease Test

- **Urea:** 20g
- **Yeast Extract:** 0.1g
- **Na_2HPo_4:** 9.5g
- **K_2HPo_4:** 9.1g
- **Phenol Red:** 0.01g
- **Distilled Water:** 1 liter

Inoculate growth from each presumed positive TSI slant culture into tubes of Urea broth inoculated tubes of Urea broth. Dissolve the ingredients in distilled water. Do not heat. Sterilize by filtration through 0.45µm membrane. Aseptically dispence 1.5 -3.0ml portions in sterile test tubes. Incubate 24 hrs at 35°c turn at purple red positive.

Identified microorganisms are compared with systemic Bacteriology (Bergey, s manual).

Antibacterial Activity

The antibacterial assay was carried out as per Selvin and Lipton(2004), Briefly the base layer was prepared with 10-15ml(1.5 % w /v) of Muller hint on agar (Himedia)four numbers of sterile porcelain beads were placed on the base layer at 60°(angle) apart. The overlaid seed layer was prepared by pouring 15ml containing 0.2ml of prepared inoculum. The porcelain beads were removed carefully with sterile forceps. The resultant wells in triplicates were filled with 20µl of the appropriate algal extract.

MINIMAL INHIBITORY CONCENTRATION

Determination of mechanisms of antibiosis (bacteriostatic or bactericidal).

The minimal inhibitory concentrations (MIC) was determined by the broth dilution method. Appropriate seaweed extract was diluted in tryptone broth or peptone water and filled up to the brim of each well and negative control were used to validate the inferences. The plates were incubated at 37°c for 24 hrs, after incubation the bioactivity was determined by measuring the diameter of inhibition zone.

INVITRO ASSAYS

DIFUSSION AGAR TEST

The method widely used to evaluate antibacterial activity is the well diffusion method. Soluble extracts diffuse into the culture medium generally Muller hint on agar used for bacteria. The plates were incubated under the optimal conditions for each microorganism, and the presence or not of an inhibition zone was determined.

Bacterial growth in the presence of algal extracts was monitered by measuring optical dencity (OD) at 490 nm every 30 min for 24 hrs.

MINIMUM BACTERICIDAL CONCENTRATION

Minimum bactericidal concentration (MBC) refers to the minimum concentration of an antimicrobial drug that causes a 3-logarrithmic decrease in the size of the standard inoculum.MBC test has been a powerful method to compare the germ -killing

activity of several antimicrobial agents at once, for screening purposes. After a completed minimum inhibited concentration (MIC) test, the MBC can be determined using a series of steps. The MBC is complementary to the MIC, while the MBC demonstrates the lowest level of antimicrobial agent results in microbial death and the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth.

Table 1: Antibacterial Activity of Seaweeds in 3 Different Solvents

		Solvent (4%)	
Marine Algae	**Acetone**	**Methanol**	**Distilled Water**
Green Algae:			
Ulva Lactuca	0.9mm	1.5mm	1.4mm
Ulva reticulata	2.1mm	1.8mm	1.4mm
Monostromalatismum	1.8mm	1.6mm	1.2mm
Codium fragile	2.2mm	1.6mm	1.2mm
Caulerpa corynephora	2.4mm	2.3mm	1.2mm
Brown Algae:			
Sargassum muticum	2.5mm	3.1mm	2.4mm
Leathesia marina	1.8mm	3.0mm	1.2mm
Padina pavonica	1.5mm	1.8mm	1.5mm
Colpomeisisinosa	1.5mm	1.8mm	1.2mm
Sargassum wightii	3.5mm	2.4mm	1.5mm
Padina tetrastromata	2.2mm	1.4mm	1.2mm
Red Algae:	2.2mm	1.6mm	1.2mm
Gelidium Spinosum	2.1mm	1.8mm	1.2mm
Gracilaria Edulis	1.8mm	2.0mm	2.1mm
Gracilariagreville	0.8mm	1.3mm	0.7mm
Kappaphycusalvarasi	3.0mm	2.3mm	0.8mm
Laurencia karachiana	1.8mm	1.5mm	1.2mm
Parphyraumblicalis Polysiphoniaceramiae formis	1.0mm	1.4mm	1.0mm

Table 2: MIC of Seaweeds Extract against E. Coli

Macroalgae	10-1	10-2	10-3	10-4	10-5	10-6
Gelidium spinosum	1.3mm	0.9mm	0.9mm	0.7mm	0.7mm	0.7mm
Gracilaria edulis	1.0mm	0.8mm	0.7mm	0.7mm	0.7mm	0.7mm
Ulva lactuca	0.9mm	0.8mm	0.7mm	0.7mm	0.7mm	0.7mm
Ulva reticulata	1.0mm	0.9mm	0.8mm	0.6mm	0.5mm	0.4mm
Sargassum muticum	1.3mm	0.9mm	0.7mm	0.5mm	0.4mm	0.2mm
Gracilariagreville	1.2mm	1.1mm	1.0mm	0.9mm	0.8mm	0.7mm
Monostromalatismum	0.9mm	0.8mm	0.7mm	0.6mm	0.6mm	0.6mm
Kappaphycusalvarasi	1.3mm	1.2mm	1.0mm	1.0mm	1.0mm	0.9mm
Codium fragile	1.1mm	1.0mm	0.9mm	0.7mm	0.7mm	0.7mm
Caulerpa corynephora	1.2mm	1.1mm	1.0mm	0.9mm	0.8mm	0.7mm
Leathesia marina	1.2mm	1.0mm	0.9mm	0.7mm	0.5mm	0.4mm
Padina pavonica	1.6mm	1.3mm	1.1mm	1.0mm	0.9mm	0.8mm
Colpomeisisinosa	1.0mm	0.9mm	0.9mm	0.7mm	0.6mm	0.6mm
Laurencia karachiana	1.8mm	1.7mm	1.6mm	1.5mm	1.1mm	1.0mm
Sargassum weightii	1.5mm	1.2mm	1.1mm	0.9mm	0.8mm	0.7mm
Parphyraumblicalis	1.0mm	0.9mm	0.8mm	0.7mm	0.7mm	0.7mm
Padina tetrastromatica	0.9mm	0.7mm	-	-	-	-
Polysiphoniaceramiaeformis	0.8mm	0.6mm	0.6mm	0.5mm	0.5mm	0.5mm

Table 3: Phytochemical Analysis of Sargassum Weightii Extract

S.No.	Test	Experiment	Observation	Inference
01.	Test for Alkaloids	Extract+Wagner's reagent	Reddish colour appear	Alkaloid present
02.	Test for Steroids	1ml test solution+chloroform+2 drops of conc.H_2 SO_4	Red colour develops	Steroids present
03.	Test for Tannins	1ml extract+1% ferric chloride+20 ml distilled water	-	Tannins absent
04.	Test for pholobatanins	10mlextract+few drops1%Hcl	Red colour deposition present	Presence of pholobatanins
05.	Test for Saponins	1ml extract +1ml distilled water &mix well	Foamy lather present	Presence of saponins
06.	Test for Flavonoids	1ml extract+few drops 1%ammonia solution	Yellow colour appears	Presence of flavanoids
07.	Test for Terpenoids	5ml extract+2ml chloroform+3ml conc.H_2 SO_4	Reddish brown colour formed	Terpenoids present
08.	Test for Cardiac Glycosides	5ml test solution +2ml glacial acetic acid+1ml ferric chloride+add 1ml of conc.H_2 SO_4	Brown ring appears	Presence of cardiac glycosides
09	Test for Phenolic compound	1ml seaweed solution+few drops of Ferric Chloride	Intense colour develops	Presence of phenol compound.
10.	Test for Aromatic Acids	1ml Seaweed solution+a pinch of Sodium bicarbonate	Formation of brick effervescence.	Presence of Aromatic acids.
11.	Test for Xanthoprotein	1ml seaweed+add a few drops of H_2 SO_4 +1ml Ammonia solution.	Yellow ppt.	Xanthoprotein present

Table 4: Phytochemical Analysis of Laurencia Karachiana

S.No.	Test	Experiment	Observation	Inference
01.	Test for Alkaloids	Extract+Wagner's reagent	Reddish colour appear	Alkaloid present
02.	Test for steroids	1ml test solution+chloroform+2 drops of conc.H_2 SO_4	Red colour develops	Presence of steroids
03.	Test for Tannins	1ml extract+1ml Distilled water+few drops of 1%lead acetate	White ppt present	Presence of Tannins
04.	Test for pholobatanins	10ml extract+few drops1%Hcl	-	Pholobatanins absent
05.	Test for Saponins	1ml extract +1ml distilled water &mix well	Foamy lather develops	Presence of Saponins
06.	Test for Flavonoids	1ml extract+few drops 1%ammonia solution	Appearance of yellow colour	Flavonoids present
07	Test for Terpenoids	5ml extract+2ml chloroform+3ml conc.H_2 SO_4	Reddish brown colour formed	Terpenoids present
08	Test for Cardiac Glycosides	5ml test solution +2ml glacial acetic acid+1ml ferric chloride+add 1ml of conc.H_2 SO_4	Brown ring appears	Presence of cardiac glycosides
09	Test for phenolic compound	1ml seaweed solution+few drops of Ferric Chloride	Intense colour develops	Presence of phenol compound.
10.	Test for Aromatic acids	1ml of seaweed solution+add a pinch of Sodium bicarbonate	Brick effervescence absent	Aromatic compound absent
11.	Test for Xanthoprotein	1ml of seaweed solution+add a few drops of H_2 SO_4 +1ml Ammonia solution.	Yellow ppt	Xanthoprotein present

RESULTS AND DISCUSSIONS

The antibacterial activity of seaweeds has been determined using pathogens. In the preliminary assay of organic solvents like Acetone, Methanol, and Distilled water. The zone of inhibition produced by these extracts against pathogenic

microorganisms is summarized in Table.1.The Acetone extract was most active and showed broader zone of inhibition against most of the pathogens. The highest zone of inhibition 3.5mm, 3.0mm was produced by Acetone extracts of Sargassum weightii, Laurenciakarachiana respectively. The 100µl extract having antibacterial activity was then tested for their potency by MIC determination assay and results were summarized in Table.2.The Acetone extract was most active and showed antibacterial activity at lower concentrations.

The extracts of Sargassum weightii, Laurenciakarachiana were effective against most of the tested pathogenic Bacteria. These results indicate significant capacity of Sargassum weightii, Laurenciakarachiana bioactive compounds phenol as antibacterial agent in the treatment of pathogenic organisms.

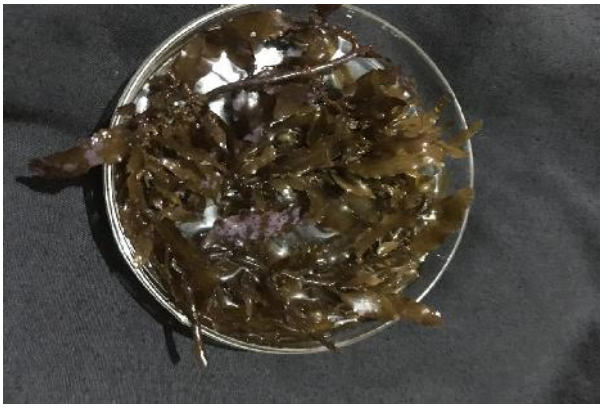

Figure 5: Sargassum Weightii (Macroalgae).

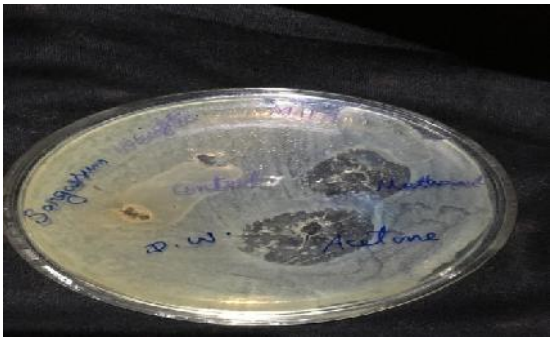

Figure 6: Sargassum Weightii Extract on Different Solvents.

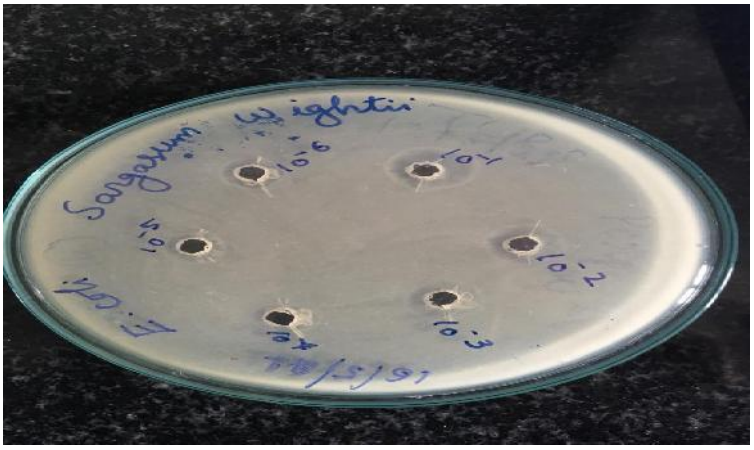

Figure 7: MIC of Sargassum Weightii Acetone Extract Against E. Coli.

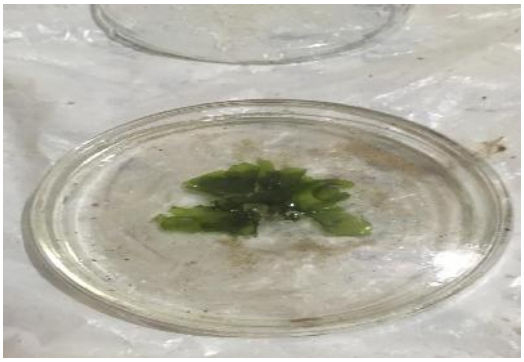

Figure 8: Ulvalactuca (Macroalgae)

Figure 9: Ulva Lactuca Extract on Different Solvents.

Figure 10: Antibacterial Activity of Ulva Reticulata against E. coli.

DISCUSSIONS

Antibacterial activity of seaweeds differs in each one species and solvent extracts also differs the antibacterial activity. Brown algae and Red algae have higher value than green macroalgae, the only reason being high potential phytochemical compounds present.

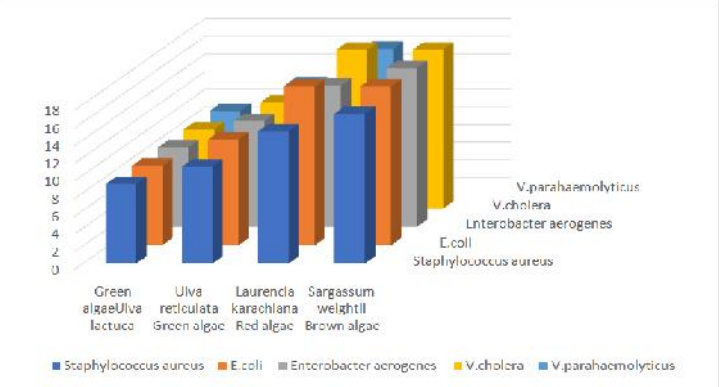

Figure 11: Antibacterial Activity of Seaweed Extracts (100µl) Against Seafood Pathogens.

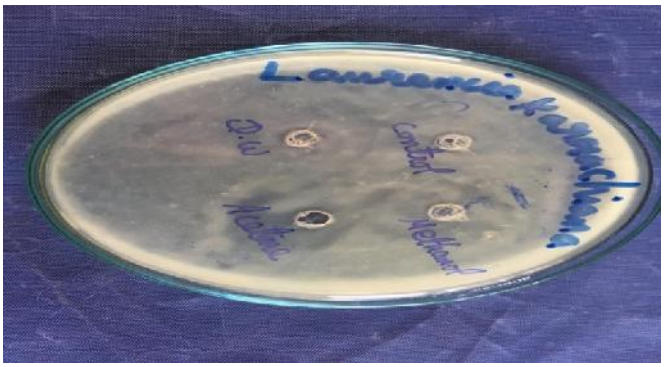

Figure 12: Laurenciakarachiana Seaweed Extract on Different Solvent.

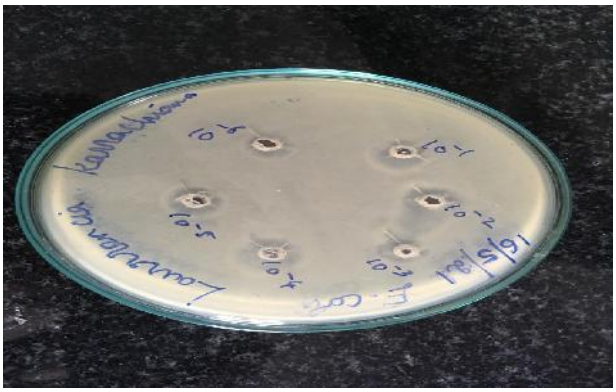

Figure 13: MIC of Laurencia Karachiana.

CONCLUSIONS

The extracts of Sargassum weightii, Laurenciakarachiana were effective against most of the tested pathogenic Bacteria. These results indicate significant capacity of Sargassum weightii, Laurenciakarachiana bioactive compounds phenol as antibacterial agent in the treatment of pathogenic organisms.

REFERENCES

1. *Ranan.N.2006 Phytochemical analysis, New India publications.p.10.*
2. *Rao.P.P.S.1991.Biological investigation of Indian marine algae, screening of some green, red, and brown seaweeds for their anti microbial activity. Seaweed Res.Util.14(1), 37-43.*

3. *Kalliaperumal.N., Methods of cultivation and Economic values of Seaweeds. In. Advances in Marine and Brackishwater Aquaculture., Springer., Newdelhi.pp., 209-216., 2015.*
4. *Neu, H.C.(1992).The crisis in antibiotic resistance. Science, 257, pp.1064-1073.*
5. *Cohen. M.L., (1992)., Epidemiology of Drug resistance :Implications for a post antimicrobial era. Science, 257.pp.1050-1055.*
6. *Jenifer. M. Andrews., Journal of antimicrobial Chemotheraphy(2001)., 48, Suppli.S.5-16.*
7. *Jevans.M.P., 1961., Celbenin-resistant Staphylococci. Brit. Med.J., 1, pp.124-125.*
8. *FAO/WHO., Guidelines on nutrition labelling. Rome.*
9. *Dr. A. Murugan, Seaweed Research and utilization Vol.40, No:1, 2018, 097-7560.*
10. *M.C.HughDJ.A guide to the seaweed industry.FAO Fishes Technical paper 2003;441:105.*
11. *Desai.B.N.Seaweed resources and extraction of alginate and agar.In proceedings of the seminar on Sea, Salt and Plants. Bhavnager;1967.p344-1.*
12. *Boopathy.N.and Katheresan.K., 2010., Anticancer drug from marine flora :An overview.J.oncology.pp1-18.*
13. *Rolinson, G.N(1961), Celbenin resistant Staphylococci. Brit. Med.J., 1, pp.125-126.*
14. *Muhammad AfzalRizhi, Comparative antibacterial activities of sea weed extracts from Karachi coast, Pakistan, Journal of pharmacology, Pakistan. July 2010, pp-53-57.*
15. *Alisha., Ritu Prakash Dubey and Aisha Haider. Seaweeds nutritional and health benefit, The pharma Innovation Journal 2019;8(8);80-83.*
16. *HerreStegenga., Walter De Gruyter., Srilankan Seaweeds methodologies and field guide to the dominant species., 54(1)., 109-110., 2011.*
17. *Argulis.L., Schwartz. K., Five kingdom an illustrated guide to the phyla of life on earth. Newyark: Freeman, WH and company, 1982, pp 16-17.*
18. *S.Felix and P.Pradeepa., (2011)Seaweed(Ulva reticulata)based fermented marine silage feed preparation under controlled conditions for Penaeus monodon Larval development.*
19. *Shilpi Gupta., Nissreen Abu-Ghannam., Recent development in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods., Innovative food Science and Emerging Technologies., Vol.12.2011, pp600-609.*
20. *Urhida., Murata.M., Fermentative preparation of single cell detritus from seaweed, Undariapinnalifida, suitable as a replacement hatchery diet for unicellular algaeAquaculture.207:345-357., 2001.*
21. *Atlas. R.M., Bartha. R., Microbialecology: Fundamentals and applications, Menlopark, CA, USA; Benjamin/Cummings., 1998.*
22. *M.D.Guiry., and G. M.Guiry., Algae base World-wide electronic publication, National university of Ireland., Galeway., Algae., 2016., June 21., http://www.algaebase.org.*

23. *J.Hou., andJin. Y., The healing power of Chinese herbs and medicinal recipes Howorth Press Inc., Newyork., 2005., pp662.*
24. *Mourtitsen.O.G., Suchi; Food for the Eye, the body, and the soul. Newyork, NY, USA; Springer Science;2009.*
25. *Ambica.S., and Sujatha., K., 2016.Organicseed treatment with seaweed nanopowders on physiological quality and enzyme activities in aged seed of Pigeon Pea. The Bio scan 11(1):352-357.1979 Antibiotic substances from marine flora.In Marine algae in Pharmaceutical Science.*
26. *Singh. A.P., and Chaudhary. B.R., 2010.Analysis and Invitro antibacterial screening of pethophoraeedogonia. Willrock-A preliminary phycochemical freshwater green alga forming Mats in the water bodies.J.Algal Biomass utln.1(2):33-41.*
27. *Trixie Erum, George G.Frias Christopher J.Cocal, Sargassum muticum as feed substitute for Broiler.Asia Pacific journal of education, Arts and Sciences Vol.4 No.4 October 2017.*
28. *Jayachandran Venkatesan, Sukumaran Anil, Introduction to Sea weed polysacchrarids, July 2017, pp 1-9.l wai and Distribution of Padina boryana Thivy Dictyotales, Phaeophyta from Myanmar*
29. *Dona Paula, Goa, Sea weeds of the central west coast National institute of Oceanography.*
30. *TsengC.K., The past, present and future of Phycology in China. Hydrobiologia, 2004;512:11-20.*
31. *Ahilan.B., Sujathkumar. N.V., Seaweeds, is it really useful, Seafood exports.Journal.1990.Vol.22, No-9-10, PP-23-25.*
32. *Fleurence.J., I. Levine., Seaweeds as food, Seaweeds in health and disease San Diego, CA, USA .In. J.Fleurence and Academic press 2016, pp, 149-167.*
33. *Romero.J., Feijoo.C.G., and Navarrete.P.2012.Antibiotics in Aquaculture -use, Abuse and Alternative s.Im:Carvalho, E.D., David.G.S., Silva.R.J.Health and Environment in Aquaculture. In Tech Croatia, pp.159-198.*
34. *K.Kolanjinathan and D.Stella, International Journal of pharmaceutical and Biological Archives.Comparative studies on Antimicrobial activity of Ulva reticulata, Ulvalactuca against human pathogens. 2011;2(6): 1738-1744.*
35. *Tarek Am Salem, Alef. M.Ibrahim Anticancer activity of Egyption marine algae Ulva regida.International Journal of health Sciences 5 (2 supple 1), 6, 2011.*
36. *Ju-iiLee., Hyung-Geunkim., Paul John Legaspi Geraldino., II-Ki Hwang and Sung Min Boo., Molecular classification of the Genus Grateloupia (Halymeniaceae, Rhodophyta)in Korea.2009., 24(4):231-238.*
37. *L.Jeyanthi Rebecca, V.Dhanalakshmi, Tina Thomas. Journal of chemical and pharmaceutical Research 4(11), 4859-4863, 2012. A Comparison between the effects of three algal extracts againgt pathogenic bacteria.*
38. *J.A.Akankali., E.I.Elenwo., Sources of marine pollution on Nigerian Coastal Resources: Anoverview. Journal of Marine Science., vol.5., 2015.*

39. *U.Thilina., Jayawardena., K.K.AsankaSanjeewa., You-Jim Jeon., Padinaboryana;a brown alga from the Maldives inhibition of α-MSH-Stimulated melanogenesis via the activation of ERK in B16 F10 cells. Fisheries and Aquatic Sciences 23, 8(2020).*

40. *Mya kyawlwai and U.Soe.Htun., Studyies on the morphology and Distribution of Padina boryanaThivyDictyotales, Phaeophyta from Myanmar. Universities Research Journal Vol, No;4, 2008.*

41. *AnusreeManeesh., Kajal Chakraborty., FasinaMakkar., Pharmacological activities of brown seaweed Sargassum wightii family Sargassaceae) using different invitro models. International Journal of Food Properties., 20(4), 931-945., 2017.*

42. *Izabela Michalak., Katarzyna Chojnacka., and Agnieszka Saeid., Plant Growth Biostimulants, Dietary feed supplements and cosmetics formulated with supercritical Co_2 Algal Extracts. Molecules., 2017., 22(1)., 66.*

43. *Andrea Gomez., Zavaglia., Miguel.A., Prietolage Cecilia Jimenez Lopez., JuancMejisto and Jesus Simai Gandara., The potential of Seaweeds as a source of functional ingredients of prebiotic and Anti oxidant value.Anti oxidants 2019., 8(9)., 406.*

44. *Perez.M.J., Falque.E. and Dominguez.H.2016.Antimicrobial action of compounds from marine seaweed.Mar.Drugs.14(52)., 1-38.*

45. *K.Murugan.K., S.S.Sumyya., S.G.Sreelakshmi., Nutraceutical value added seaweed-Gracilaria corticate var.cylinrica from Thoothukudi., 2018;11(2)., 97-100.*

46. *Mnilal.A., Sujith.S., Selvin.J., Kiran.G.S., Shakir.C., Gandimathi.R., and Pankkar.M.Y.N.2009.Biopotential of seaweeds collected from south west coast of India.J.Mar.Sci.Tech.17:67-73.*

47. *Nanthinidevi.K., Ajith Kumar.T.T., and Balasubramanian.T.2014.Antibacterial and antioxidant effects from seawed, Sargassumwightii(Greville, 1848) against marine ornamental fish pathogens.J.Coastal Life Medicine.2(10):773-783.*

48. *Natrah.F.M.I., Harah.M., JaparSidik.B., Izzatul.N.M.S., and Syahidah.A.2015.Antibacterial activities of selected seaweed and sea grass from port Dickson coastal water against different aquaculture pathogens Sains Malaysiana.44(9):1269-1273.*

49. *N.R..NailaBanu, M.MathevanPillai, S.Ponrasika and J.Anusha., Astudy of epiphytic diatoms and their adhesive strength on Chlophycean seaweeds in Chinnamuttom near Kanniyakumari, Tamilnadu.*

50. *R.Sreerekha and K.Padmakumar, Distribution of diatoms in relation to physico-chemical parameters in the Coastal waters of Valiyathura, Thiruvananthapuram, southwest coast of India.*

51. *S.Rameshkumar, R.Rajaram, T.Muralisankar and N.Manickam, Evaluation of antioxidant capacity of red algae Kappaphycusalvarezii from Mandapan coast southest coast of India.*

52. *Sankarammal, C., G.Rajakumari, V.Selvakumar, M.Elangovan, J.IndhuraPriadharsani, M.Kalaiselvam and A.Murugan, Nutritional and health benefit s of seaweeds.Quantitative sensory evaluation of functional foods from Gracilaria edulis.*

53. *A.Jenifer and K.Sathyamurthy., Nutraceuticals from algae on Alzheimer's disease-An overview.*
54. *V.Sornalakshmi, P.S.Tresina, K.Paulpriya and V.R.Mohan, Seaweed Research &Utilisation vol.40(1)June 2018, pp.35-41.FTIR analysis and antioxidant activity of marine seaweed Acanthophoraspicifera (M.Vahl) Borgesen.*
55. *N.R.LailaBanu, M.Mathevanpillai, S.Ponrasika and J.Anusha., Community structure of epiphytic diatoms and their adhesive strength in the collected seaweeds of Muttapathi, Kanniyakumaridistrict, Tamilnadu, India.*
56. *Archana Pal., Mohit Chandra Kamthania., Ajaykumar., Bioactive compounds and properties of seaweeds:AReview.Marine Science., vol.4(24)2014.*
57. *Gupta.S., andAbu.Ghannam.N., (2011)Bioactive potential and possible health effects of edible brown seaweeds.Trends in Food Science &Technology, 22(6), 315-326.*
58. *Alish, RiluPrakash, Dukey and Aisha Haider, The pharma Innovation, Journal 2019;8(8);80-83.Seaweed Nutritional and health benefits.*
59. *K.Jayaprakash., N.Srikumaran., and Swarnakala.Seaweed research in India -A noveldomainin marine Biotechnology.International Journal of pharmaceutical in marine sciences and Research.Vol.12, June 2021.*
60. *Yuvaraj, N., Kanmani.P., Satishkumar.R., Paari.A., Pattukumar.V and Arul.V.Extraction purification and partial characterization of Cladophora glomerata against multidrug resistant human pathogenAcinetobacterbaumanni and fish pathogens World J.Fish Mar.Sci.3:51-57., 2011.*
61. *Vinayak.C., Sabu.S., and Chatterjii.A.2011. Bio-prospecting of afew brown seaweeds for their cytitoxic and antioxidant activities, Evidence -based comple Alt.Med.pp.1-9.*
62. *Tuney.I., Cadirci.B.H., Unal.D and Sukatar.A., 2006 Antibacterial activities of the extracts of marine algae from the coast of Urla(Ismir., Turkey)Turkish j.Biol.30:171-175.*
63. *Santhanam ShanmughaPriya, AseerMnilal, Kalimuthusamy, Natarajaseenivasan, Annals of Microbiology 58, 535-541(2008)., Antimicrobial activity of seaweeds extracts against multiresistant pathogens.*
64. *Selvin.J., Lipton.A.P., (2004)Biopotentials of Ulva fasciata and Hypneamusciformis collected from the Peninsular coast of India.Journal of Marine Science and Technology, 12:1-6.*
65. *N.Jothinaya., C.Anbazhagan., Effect of Seaweed liquid fertilizer of Sargassumwightii on the growth and biochemical characteristics of Abelmoschus esculentus (L).Medikus., Recent Research in Science and Technology1(4)., 155-158., 2009.*
66. *Lima-Filho J.V.M., Carvalho.A.F.F.U., Freitas.S.M.(2002), Antibacterial activity of extracts ofsix macroalgae from the Northeastern Brazilliancoast, Brzilian Journal of Microbiology, 33:311-333.*
67. *Masuda.M., Abe.T., Sato.S.(1997)Diversity of halogenated secondary metabolites in the red alga(Rhodomelaceaeceramiales)Journal of Phycology., 33:196-208.*
68. *MariaJosePerez, ElenaFalque and Herminia Dominguez., 2016, MarineDrugs., 14(3)., 52.*

69. *Aubert.M., Aubert.J., and Gauthier.M., 1979, Antibiotic substances from marine flora.InMarine algae in pharmaceutical Science Heinz A.Hoppe., Torelevring., Yukio Tanaka(Eds)., Walter de Gruyter.Newyark.pp., 267-292.*

70. *N.Vijayanand., S.Sivasangari Ramya., S.Rathinavel., Potential of liquid extracts of Sargassum wightii on growth, biochemical and yield parameters of cluster bean plant.Asian Pacific Journal of Reproduction., 2014;3(2):150-155.*

71. *Bauer., A.W., Kirby.W.M.M., Sherris.J.C.T., and Truck.M., 1966.Antibiotic susceptibility testing by a standardized single disk method.American J.Clinical Pathology.45(4):493-496.*

72. *Burridge.L., Weis.J.S., Cabello.F., Pizarro.J.and Bostick.K.2010.Chemical use in Salmon aquaculture a review of current practices and possible environmental effects.Aquaculture.306(1):7-23.*

73. *Caccmese.S., Toscano.R.M.Furnari.G.and Cormaci.M., 1985., Antimicrobial activities of red and brown algae from southern Italy Coast.Botanica Marina.28(11):505-507.*

74. *Sreenivasan.R., Indu.H., Archana.G., andGeetha.S., The antibacterial activity of some marine algae from south east coast of India.J.pharmacy.Res.3:1907-1912.*

75. *S.Sivasangari Ramya., S.Nagaraj., N.Vijayan., Biofertilizing Efficiency of brown and green algae on growth, biochemical and yield parameters of Cyamopsis tetragonolaba(L).Taub.Recent Research in Science and Technology., 2(5)., 42-52., 2010.*

76. *Sheler.P.S., Reddy.V.K., Shelar.G.S., Kavitha.M., Praveenkumar.G.G., andReddy.G.V.S., Medicinal value of seaweeds and its applications -a review.ContinentalJ..Pharmacology and ToxicologyRes.5(2):1-22., 2012.*

77. *Shimaa.M., Shafay.E., Ali.S.S., and El-Sheekh.M.M., 2016 Antimicrobial activity of some seaweeds species from Red Sea, against multidrug resistant Bacteria.Egyptian J.Aquatic Res.42:65-74.*

78. *Taskin.E., Ozturk.M., Taskin.E., and Kurt.O.2007.Antibacterial activities of some marine algae from the Aegean Sea(Turkey).AfricanJ.Biotech.6(24):2746-2751.*

79. *V.Sornalakshmi., P.S.Tresina., K.Paulpriya and V.R.Mohan., FTIR analysis and antioxidant activity of marine SeaweedAcanthophoraspicifera (M.Vahl)Borgesen., Seaweed Research and utilization.Vol.40(1)pp.35-41., 0971-1550., 2018.*

80. *Chennubhtla., V.S.Krishnamurthy., and Rao.K.S., andKaliapeumal.N., Effect of some adverse factors on the diversity and distribution of marine macroalgae along the Indian Coasts.Seaweeds Research and Utilization 36(1).pp.5-11., 2015.*

81. *Thanigaivel.S., Hindu Vidya.S., Vijayakumar.S., Mukherjee.A., Chandrasekaran.N. and Thomas.J., 2015 Differential solvent extraction of two Seaweeds and their efficacy in controlling Aeromonas salmonicida infection in Orechromismossambicusanovel therapeutic approach.Aquaculture.433:56-64.*

82. *Thompson.J.E., Walker.R.P. and Faulkner.D.1985.Screening and bioassays for biologically active substances from forty marine sponge species from San Diego, Californa.USA.Ma.Bio.88(1):11-21.*

83. *Choudhury.S., Sree.A., Mukherjee.S.C., Pattnaik.P., andBapuji.M.2005.Invitro antibacterialactivity of extracts of selected marine algae and Mangroves against fish pathogens.Asian Fish.Sci.18:285-294.*
84. *Cox.S., Ghannam.N., and Gupta.S.2010 AN assessment of the antioxidant and antimicrobial activity of six species of edible Irish Seaweeds.Int.Food Res.J.17:205-220.*
85. *Elsie.B.H., Sudha.P.N., and Dhanarajan.M.S.2010.Invitro study on antibacterial activity and phytochemical analysis of marine algae (Seaweeds)Sargassum whilliGracilaria edulis using three different solvent extracts.TheEcoscan 4(4):343-345.*
86. *Gomez.V., Mercado, S., Rivas.G., and Sanchez.A.2010.Antibacterial and anticancer activity of seaweds and bacteria associated with their surface.Revista de Biologia marina Oceanografia 45:267-275.*
87. *Gupta.S., Rajauria.G., and Abu-Ghannam.N.2010 Study of the microbial diversity and antimicrobial properties of Irish edible Brown seaweeds. Int.J.Food Sci.Technol.45(3):482-489.*
88. *Kolanginathan.K., Ganesh.P., and Govindarajan.M., 2009.Antibacterial activity of Etanol extracts of Seaweeds against fish bacterial pathogens European Rev. for Medical and Pharmacological Sci.13:173-177.*
89. *Lavanya.R., and Veerappan.N.2011.Antibacterial potential of six seaweeds collected from Gulf of Mannar of southeast coast of India.Adv.Biol.Res.5:38-44.*
90. *Manilal.A., Sujith.S., Sabarathnam.B., Kiran.G., Selvin.J., Shankir.C.and Lipton.A.2010.Bioactivity of the red alga Asparagopsistaxiformis collected from the south western coast of India.Braz.J.Ocenog.58(2):93-100.*
91. *Ikkyochung, Calvyn.F.A., Sandak and John Bear dall., The future of seaweed aquaculture in a rapidly changing World Eurupean Journal of Phycology.Volume 52, 2017.*
92. *Ibrahim Rahimi, AmirShakerian&AmirGhasemiFalavarjani, Prevalence and antimicrobial resistance of Salmonella isolated from fish, shrimp, Lobster and Crab in Iran.Comparative clinical pathology, 22, 59-62(2013).*
93. *Nellemann.C., Corcoran.E., Duarte.C.M., Valdes.L., De young.C., Fonseca.L.andGrimsditch.G.(2009)Blue Carbon.A rapid response assessment GRID-Arendal:United nations Environment programme.*
94. *Brinda.P., Sasikala., Purushoth.K., Preliminary phytochemical studies of higher plants.Etanobotony, 1966:36:493-496.*
95. *MunawwarRasheed., Rabia Bibi., ShahnaRashid., andDr.ValeriaCassanoRosangela da Silva.New Seaweed Spies discovered along Karachi Coast Laurencia karachiana.International Journal of Plant taxonomy.Feb 13, 2020.*
96. *Bibi.R.;Cassano.V.DaSilva Medeiros.R., Rasheed.M., 2019;Morphological and Molecular systematic investigation of Laurencia karachianasps from Karachi, Pakistan.Phytotaxa.404(1);23-40(8).*
97. *RatihPangestuti., Evi Amelia Siaharan., S-kwon kim., Photoprotective substances derived from marine algae.Marine Drugs. 16(11), 399, 2018.*
98. *RatihPangestudi., Evi Amelia Siaharan., Se-kwonkim., Photoprotective substances derived from marine algae.Marine Drugs.16(11), 399, 2018.*

99. *Boney E(1965)Aspects of the biology of the seaweeds of economic importance.Adv.Mar.Bio.3:105-253.*

100. *DaveM.J., Parekh.R.G., Ramavat.B.K., Doshi.Y.A.and Chauhan.V.D(1987)Protein content of red seaweeds from Gujarat coast.Seaweeds Re.Utiln.10(1):17-20.*

101. *Chaturvedi.D.K., Varma.S.S., andKharesp (1979)Studies on feeding marine algae (Gracilaria meal)in laying white leghorn birds proc.(Abstract)Int.seaweedsymp.Marine Algae of the Indian Ocean region.CSMCRI, Bhavnager, India:pp 51.*

102. *P.V.Subba Rao., C.Periasamy., K.Sureshkumar., A.Srinivasa Rao and P.Anantha Raman., 2018.Seaweeds :Distribution, Production and uses.*

103. *MitaliPriyadarsiniPati., Satyabratadassharma., Lakshman Nayak., Chita Ranjan Panda., Uses of seaweed and its application to Human welfare :A review, International Journal of Pharmacy and Pharmaceutical Sciences.Vol 8, 2016, 0975-1491.*

104. *Mayer.A.M.S., LehmannV.K.B., Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antihelmintic, antiplatelet, antiprotozoal, and antiviral activities;with actions on theCardiovascular, endocrine, immune and nervous systems;and other miscellancous mechanisms of action.Pharmacologist 2000;42-62(9).*

105. *ChauhanV.D., Krishnamurthi.V., An estimate of algin-bearing seaweeds in the Gulf of Kutch.Curr.Sci.1968;37;648.*

106. *ElhafiNabli., B.Jha., A.Hartmann., Impact of Seaweeds on agricultural crop production as biofertilizer.International journal of Environmental Science and Technology.14(5)., 1119-1134., 2017.*

107. *S.Sivasangari Ramya., N.Vijayanand., S.Rathinavel., Foliar application of liquid biofertilizer of brown alga Stoechospenmum marginatum on growth, biochemical and yield of Solanummelongena.International Journal of Recycling of organic waste in Agriculture 4(3), 167-173., 2015.*

108. *T.Suthin Raj., P.Nishanthi., K.Hane Graff and H.Annsuji., International Journal of Tropical Agriculture., Vol.36., 2018.0254-8755.Seaweed extract as aBiostimulant and a pathogen controlling agent in plants.*

109. *Jang.K.Kim., CharlesYarish., Eunkyoung., Miseon Park., Youngelaekim., Seaweed aquqculture :cultivation technologies, challenges and its ecosystem services., Algae., 2017., 31(1):1-13.*

110. *Xie., E.Y.Liv.D.C., Jia.C., Chen.XL., andYong.B., Artificial seed production and cultivation of the edible brown alga Sargassum naozhouense Tseng el Lu.J., 2013., Appl.Phycol.25:513-522.*

111. *Guiry.M.D., M.J.Costello., etal., Macroalgae of Rhodophycota, Chlorophycota, Chlorophycota, and two genera of Xanthophycota., 2001., Europeanregister of marine species:a check list of the marine species in Europe and a bibiliography of guides to their identification, collection patrimoines., Naturels., 50:pp., 20-38.*